CLAIMS

1/ The use of a membrane fraction of Gram-negative bacteria, comprising proteoglycans, for preparing a pharmaceutical composition which is immunostimulant and/or which is capable of inducing an antitumor immune response.

2/ The use as claimed in claim 1, characterized in that the membrane fraction comprises a membrane fraction of Klebsiella *pneumoniae*.

3/ The use as claimed in claim 1 or 2, characterized in that the membrane fraction comprises at least membrane fractions of two different strains of bacteria.

4/ The use as claimed in one of claims 1 to 3, characterized in that the membrane fraction is prepared using a method comprising the following steps:

a) culturing said bacteria in a culture medium which allows their growth, followed by centrifugation of said culture;

b) where appropriate, deactivation of the lytic enzymes of the bacterial pellet obtained in step a), then centrifugation of the suspension obtained;

c) extraction and elimination of the non-membrane-bound proteins and of the nucleic acids of the pellet obtained in step a) or b) with at least one cycle of washing the pellet in an extraction solution;

d) digestion of the membrane pellet obtained in step c) in the presence of protease enzymes, followed by centrifugation;

e) at least one cycle of washing the pellet obtained in step d) in a physiological solution and/or in distilled water; and

f) ultrasonication of the pellet obtained in step e).

5/ The use as claimed in one of claims 1 to 3, characterized in that the membrane fraction is prepared using a method comprising the following steps:

a) culturing of said bacteria in a culture medium which allows their growth, followed, where appropriate, by centrifugation;

b) freezing of the culture medium or of the pellet obtained in step a), followed by thawing and drying of the cells;

c) elimination, using a DNase, of the nucleic acids from the dried cells obtained in step b), which have been resuspended;

d) grinding of the cells obtained in step c) and clarification of the suspension obtained;

e) precipitation, in acid medium, of the suspension obtained in step d) and elimination of the pellet;

f) neutralization of the supernatant obtained in step e) containing the membrane suspension, followed by dialysis and concentration of the membrane suspension; and

g) sterilization of the concentrated membrane suspension obtained in step f).

6/ The use as claimed in one of claims 1 to 5, characterized in that the pharmaceutical composition also comprises an agent for vehiculing said membrane fraction in a form which makes it possible to improve its stability and/or its

immunostimulant activity and/or its capacity to induce an antitumor immune response.

7/ The use as claimed in claim 6, characterized in that said agent is of the oil-in-water or water-in-oil emulsion type.

8/ The use as claimed in claim 6, characterized in that said agent is in the form of a particle of the liposome, microsphere or nanosphere type, or any type of structure which enables said membrane fraction to be encapsulated and presented in particulate form.

9/ The use as claimed in one of claims 1 to 8, characterized in that the pharmaceutical composition also comprises an agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions.

10/ The use as claimed in claim 9, characterized in that the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a cytokine.

11/ The use as claimed in claim 9, characterized in that the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a regulatory agent chosen from hormones.

12/ The use as claimed in claim 9, characterized in that the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a regulatory agent chosen from growth factors.

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13/ The use as claimed in claim 9, characterized in that the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a cellular compound.

14/ The use as claimed in claim 13, characterized in that said cellular compound is a nucleic acid chosen from DNAs and RNAs.

15/ The use as claimed in claim 13, characterized in that said cellular compound is a compound of the ribosome family.

16/ The use as claimed in claim 13, characterized in that said cellular compound is a protein of the heat-shock protein family.

17/ The use as claimed in one of claims 1 to 16, for preparing a pharmaceutical composition intended to be administered in combination with an anticancer treatment.

18/ The use as claimed in claim 17, characterized in that the anticancer treatment is chemotherapy and/or radiotherapy.

19/ The use as claimed in either of claims 17 and 18, for preparing a pharmaceutical composition intended to be administered simultaneously with, separately from or spread out over time with the anticancer treatment.

20/ The use as claimed in claim 19, characterized in that the pharmaceutical composition is administered via the enteral or parenteral route.

21/ The use as claimed in one of claims 17 to 20, characterized in that said combined anticancer treatment is a chemotherapeutic treatment comprising a protease inhibitor or a compound with anti-angiogenic activity.

22/ The use as claimed in one of claims 1 to 21, for preventing and/or treating cancers.

23/ The use as claimed in claim 22, for preventing and/or treating bladder cancers, prostate cancers, colon cancers, liver cancers and malignant melanomas.

24/ A pharmaceutical composition comprising a membrane fraction of Gram-negative bacteria, comprising proteoglycans, which can be obtained using a method for preparing a membrane fraction as described in claim 4 or 5.

25/ The pharmaceutical composition as claimed in claim 24, characterized in that said Gram-negative bacterium is Klebsiella *pneumoniae*.

26/ The pharmaceutical composition as claimed in claim 24 or 25, characterized in that it is combined with an anticancer treatment by chemotherapy and/or by radiotherapy.

27/ The pharmaceutical composition as claimed in claim 26, characterized in that it contains an anticancer compound as a combination product for use which is simultaneous, separate or spread out over time.

28/ The pharmaceutical composition as claimed in claim 27, characterized in that said anticancer compound

is chosen from protease inhibitors or from compounds with anti-angiogenic activity.

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